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Effects of aquaria- and pond noise on hearing sensitivity in fish

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Abstract

Several studies on fishes showed that behaviour and auditory sensitivity may be often affected by underwater noise. The current study concentrates on holding conditions encountered by fish kept for leisure in aquaria and ponds. Noise spectra showed that all aquarium filters measured created a high amount of low frequency noise while the water outflow above the surface created additional high frequency noise components. Audiograms of a hearing specialist, the goldfish *Carassius auratus* were determined between 0.1 and 4 kHz using the non-invasive auditory evoked potential (AEP) recording technique. The amount of masking was determined in the presence of four different noise-types: aquaria with internal filter with outflow below the water surface (114 dB re 1 µPa broadband $L_{Leq,\ 1\ min}$), external filter with outflow above the water surface (119 dB), external filter with outflow below the water surface (115 dB) and an unfiltered pond (95 dB). The goldfish's hearing was masked by all filter noise types and most affected at 0.1 and 0.3 kHz by the external filter noise (threshold shifts of 15-19 dB). Pond noise had no effect on the hearing threshold relative to quiet lab conditions. The results indicate that hearing specialists are considerably masked under holding conditions found in aquaria but probably not in ponds. Thus, using a quieter filter setup with a quiet outflow might help to improve holding conditions in aquaria without compromising aeration of the water.

Keywords: Sound pressure level; Aquaria noise; Hearing; Fish; Auditory Evoked Potentials, Masking

1. Introduction

Sound is an important means of communication in aquatic environments, because it can be propagated five times faster than in air and it is not attenuated as quickly as other signals. There are numerous noise sources in the underwater environment, and some information is available about the effects of noise on hearing thresholds of species with different auditory capacities.

Fish live in an environment where the acoustic background highly varies due to the influence of currents, waves, the prevailing weather conditions, and others. This ambient noise is the ubiquitous acoustic background consisting of abiotic (wind, waves, rain, surf) and biotic (animal vocalizations, feeding sounds) sources (Hawkins and Myrberg, 1983). Many fish species are able to produce sounds via numerous mechanisms. Acoustic signals of fish have a certain stability in their amplitude, temporal and frequency characteristics. In order to facilitate intraspecific acoustic communication, the ear of fish should be specialized on hearing signals in different background noises. The knowledge about the ability of fish to discriminate signals from noise allows an understanding of the mechanism of adaptation of this sensory organ, and it is important for the investigation of mechanisms that process acoustic information (Sorokin, 1989; Popper and Fay, 1993).

Several studies on fishes showed that behavior and auditory sensitivity can be affected by underwater noise (Myrberg, 1990). Fish are exposed to a wide range of waterborne, anthropogenic noise both, in natural, and in cultured conditions. In natural aquatic environments, noise is generated by machinery, propulsion systems of large ships and by-flow. Other sources of sounds are air guns, air craft, sonic booms, sonar systems, shock tests, boat repairs, underwater explosions, auto traffic and other human activities.

An even greater amount of noise is generated in an aquaculture environment as aquaculture systems continue to intensify. Intensification requires the use of aerators, air and water pumps, tractors, harvesters, water circulation, feeding and maintenance machinery.

Anthropogenic noise may affect the behavior in several taxa with direct or indirect consequences on acoustic communication, their ecology and fitness (Wysocki and Ladich 2006, Vasconcelos et al. 2007). Consequently, fish in aquaculture facilities are chronically exposed to noise levels that are well within the hearing range of many aquaculture species.

Banner and Hyatt (1973) first analyzed the effects of such noise on eggs and larvae of two estuarine species, *Cyprinodon variegatus* and *Fundulus similis*. These authors showed that a 20 dB increase of sound level in the 40 to 1000 Hz frequency range caused reduced viability of eggs and larvae in *C. variegatus*. Also, Lagardère (1982) reported that chronic elevation of in-tank noise levels resulted in significant reduction of growth and reproduction rates, increased aggression (cannibalism) and mortality, decreased food uptake, and higher metabolic rates, expressed as ammonia excretion rate and oxygen consumption in brown shrimp, *Crangon crangon*. Terhune et al. (1990) showed that noise levels may influence Atlantic salmon smolting rates in tanks. There was a general tendency for smolting rates to be higher in fiberglass than in the noisier concrete tanks...

Noise exposure can have various effects on fishes, among them temporary hearing loss (Scholik and Yan, 2001; Amoser and Ladich, 2003; Smith et al., 2003; Popper et al., 2005), impaired sound detection and temporal resolution ability (Wysocki and Ladich, 2005a, b), damage to the sensory epithelia of the inner ear (Hastings et al., 1996; McCauley et al., 2003), and endocrinological stress responses (Smith et al., 2003; Wysocki et al., 2006).

Artificial holding conditions may be noisier than natural habitats in many cases. In holding tanks, high frequency underwater noise is produced mainly by oscillating and collapsing air bubbles, electric generators, electric air and filter pumps while low frequency noise is mainly generated by water flows, ground vibrations, aquaria wall vibrations and electrical pumps (Bart et al., 2001; Davidson et al., 2007).

Potential effects on fishes are likely to depend on the characteristics of the sound including level, duration, and the spectrum, as well as on the hearing abilities of the fish species. Fishes depend on their hearing system to get information for acoustic orientation, about prey and predators, and to communicate inter- and intra-specifically, i.e. for mate attraction, agonistic encounters and territorial defence (Hawkins and Myrberg, 1983; Hawkins, 1993; Ladich and Myrberg, 2006; Myrberg and Lugli, 2006). Therefore it is important for the well-being of aquarium fish to minimize noise levels (Kratochvil and Schwammer, 1997).

The major goals of the present study were (1) to measure and describe aquaria noise types as compared to background noise in a pond (with no water maintaining device) and (2) to investigate the effects of noise on hearing sensitivity in a hearing specialist. A hearing specialist was chosen because prior studies showed that noise had major masking effects on specialists and only minor ones on hearing non-specialists such as perciforms and salmoniforms (Amoser and Ladich 2005; Wysocki and Ladich, 2005a; Wysocki et al. 2007b). (3) A final goal of the study was to find better acoustical holding conditions for fishes kept for leisure.

The goldfish *Carassius auratus* (Cyprinidae) was chosen because its hearing abilities have been well characterized in numerous studies. Although the goldfish inhabits originally quiet waters it is often kept and bred under semi-natural or artificial holding conditions. It is therefore crucial to understand how these holding conditions noise influences its auditory perception.

2. Materials and methods

2.1. Animals

The test subjects were six goldfish *Carassius auratus* (92-128 mm standard length (SL), 20-60 g body weight (BW) from a pond near Vienna. All animals were kept in planted aquaria whose bottoms were covered with sand, equipped with half flower pots as hiding places, filtered by external filters, and maintained at a 12L:12D cycle. The fish were fed commercially prepared pond or flake food (Tetrapond or Tetramin®). No submerged filters or air stones were used in order to reduce noise in the holding tanks. Background noise in the holding tanks ranged from 112-117 dB L_{Leq}. All experiments were performed with the permission of the Austrian Commission on Experiments in Animals (GZ 66.006/2-BrGT/2006).

2.2. Noise recording and sound pressure level measurements

The different noise types were recorded using a DAT recorder (Sony TCD-D100, Sony Corporation, Tokyo, Japan). Representative sound pressure level (SPL) values of lab, pond, and aquaria noise types were measured using a sound level meter (Brüel and Kjær 2238 Mediator) and the hydrophone (Brüel and Kjær 8101, Nærum, Denmark; frequency range: 1 Hz-80 kHz, ±2 dB; voltage sensitivity: -184 dB re. 1 V/µPa), both connected to a power supply (Brüel and Kjær 2804). For that purpose the *L*- weighted (5 Hz-20 kHz) equivalent continuous SPL (L_{Leq}) averaged over 1 min of measuring time was determined. The L_{Leq} is a measure of the averaged energy in a varying sound field and is commonly used to assess environmental noise (ISO 1996, 2003). The whole system was calibrated using a Brüel and Kjær 4229 calibrator.

Aquaria noise was recorded in an aquarium at the animal keeping facilities in the Department of Behavioural Biology at the Biocenter in Vienna. The aquarium was 1 x 0.5 x 0.5 m in size with approximate 200 l of freshwater and sand on the bottom (Fig. 1, Fig. 2). It was placed on 2 cm of styrofoam, a 3 cm wooden board and a metal frame. The hydrophone was placed in the middle of the aquaria. An external (Eheim Ecco 2232) and an internal filter (Eheim Aquaball 2212) with variable water outflow were tested.

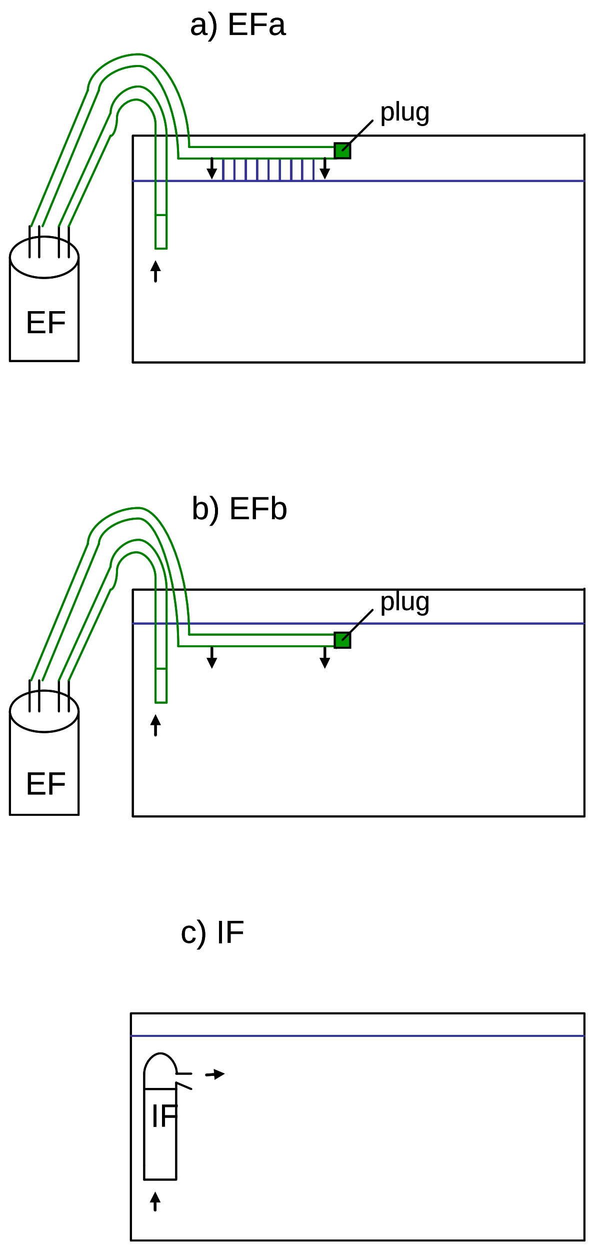

Fig. 1. Filter and water flow setups: a) EFa - external filter with outflow above water surface, b) EFb – external filter with outflow below surface, c) IF - internal filter with outflow below surface

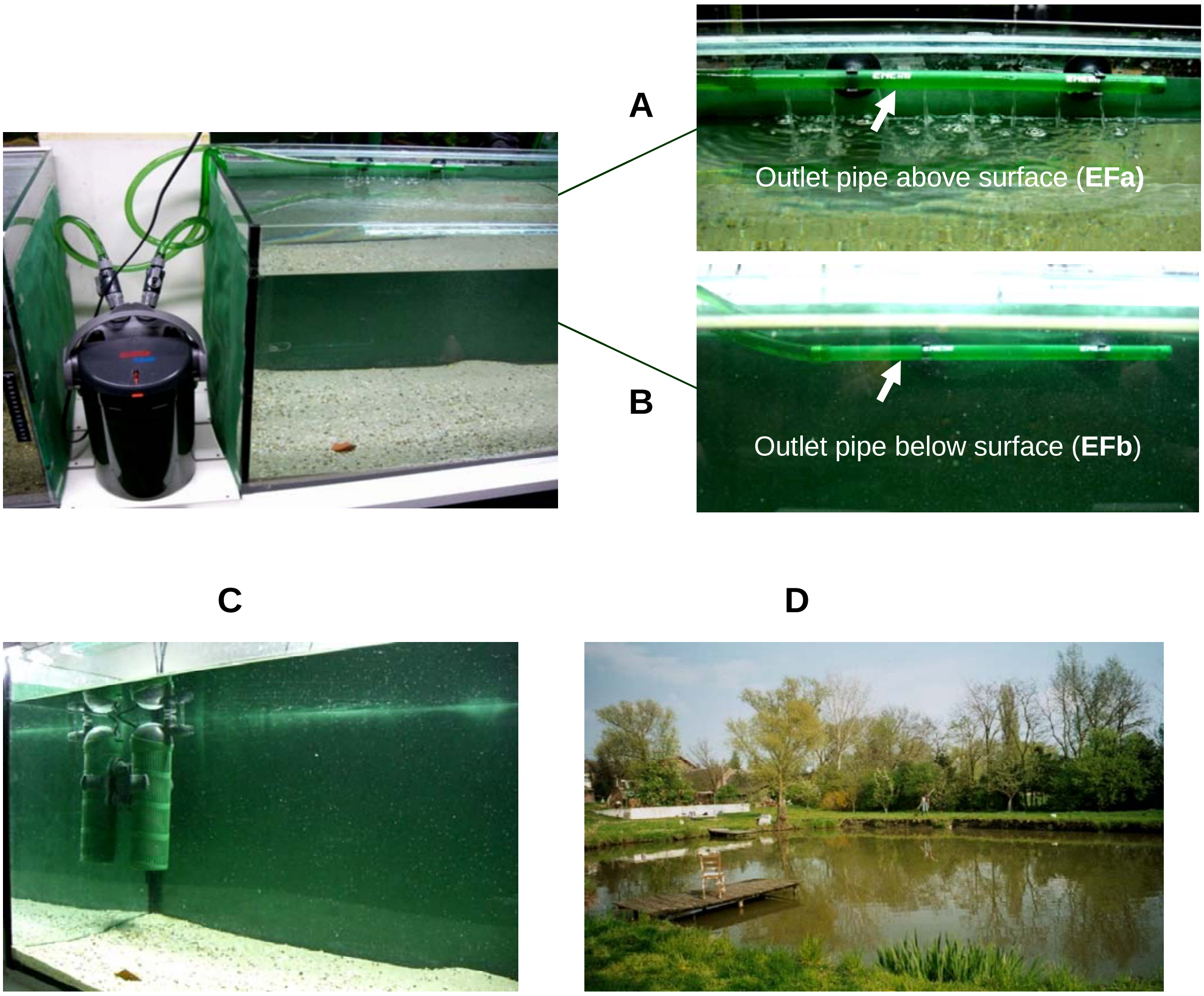

Fig. 2. Photographs of the aquaria with different filter types and water outflow setups (A-C) and of the pond (D). A: EFa - external filter with outflow above water surface, B: EFb - external filter with outflow below surface, C: IF - internal filter with outflow below surface, D: P - pond

The SPL of the internal filter with the outflow below the water surface with differing outflow rate (minimum and maximum) was also measured.

The pond is located in Prellenkirchen southeast of Vienna (geographical position: 48.1°N, 17.0°E; altitude: 163 m above sea level), measures 32 x 22 m in size with an approximate depth of about 1.8 m is populated by cyprinids. The ambient noise was a mixture of biological activity, natural water flow because of wind noise and small surface waves characteristic for summer-season. The underwater noise was recorded in 2005, 29th of July at two different places. It was a warm (water-temperature: 26.2 °C) and slightly windy day. The hydrophone was positioned approximately 0.5 m below the surface. Before and after each noise recording, the SPL (L_{Leq}) of the ambient noise was measured and then averaged.

Lab noise was recorded in the water tub, where the AEP recordings had taken place.

2.3. Noise spectra calculations

All noise recordings (sampling frequency of 44.1 kHz) were analysed using the acoustic analysis software S_TOOLS-STx 3.7 (Acoustics Research Institute, Austrian Academy of Sciences, Vienna, Austria). Averaged sound spectra of pond, aquarium and lab noise were calculated according to Amoser et al. (Amoser et al., 2004) and Wysocki and Ladich (Wysocki and Ladich, 2005a). For the calculation of absolute spectra, fast Fourier transforms (FFTs; filter bandwidth 1 Hz) for each recording were averaged and absolute spectra calculated using the L_{Leq} measured immediately before or after the recordings. These spectra were then exported as ASCII Files and imported into EXCEL, and the relative spectral values were transformed to linear values using:

$$A_i = 10\text{^}(a_i/10),$$

where A_i are the linear spectral amplitude values and a_i the logarithmic spectral amplitude values. From these values, the relative root-mean square (rms) was calculated as follows:

$$e = 10 * \log \Sigma A_i,$$

where e is the relative rms value calculated from the spectral amplitudes. The relative rms was then equalled to the absolute L_{Leq} measured with the sound level meter immediately before or after the recording, and the relative spectral levels were recalculated into absolute spectral levels.

2.4. Auditory evoked potential recordings

The auditory evoked potential (AEP) recording protocol followed was developed by Kenyon et al. (1998) and modified by Wysocki and Ladich (2005a, b). During the experiments, the fishes were mildly immobilized with Flaxedil (gallamine triethiodide; Sigma-Aldrich, Vienna, Austria). The dosage used was 0.88 ± 0.25 µg g^{-1}. This dosage allowed the fishes to retain slight opercular movements during the experiments but without significant interference of myogenic noise. Test subjects were secured in a bowl-shaped plastic tub (diameter: 33 cm, water depth: 13 cm, 1.5 cm layer of sand) lined on the inside with acoustically absorbent material (air-filled packing wrap) in order to reduce resonances and reverberations (Fig. 3.; for the illustration of the effect, see Fig. 1 in Wysocki and Ladich 2002). Fishes were positioned below the water surface (except for the contacting points of the electrodes, which were up to 1 mm above the surface) in the center of the plastic tub. This position was selected because it provided the most convenient way of placing the electrodes and because control experiments yielded no significant difference in hearing thresholds when the fishes were positioned 3 cm below the surface or in this position (Wysocki and Ladich, 2005a). A respiration pipette was inserted into the fish's mouth and respiration was achieved through a simple temperature-controlled (21-25 °C), gravity-fed water system. The AEPs were recorded by using silver wire electrodes (0.25 mm diameter) pressed firmly against the skin. The portion of the head above the water surface was covered by a small piece of Kimwipes® tissue paper to keep it moist and to ensure proper contact during experiments. The recording electrode was placed in the midline of the skull over the region of the medulla and the reference electrode cranially between the nares. Shielded electrode leads were attached to the differential input of an a.c. pre-amplifier (Grass P55C, Grass Instruments, West Warwick, RI, USA; gain 100x, high-pass at 30 Hz, low-pass at 3 kHz). A ground electrode was placed in the water. The plastic tub was positioned on an air table (TMC Micro-g® 63-540, Technical Manufacturing Corporation, Peabody, MA, USA), which rested on a vibration-isolated concrete plate. The entire setup was enclosed in a walk-in soundproof room, which was constructed as a Faraday cage (interior dimensions: 3.2 m x 3.2 m x 2.4 m).

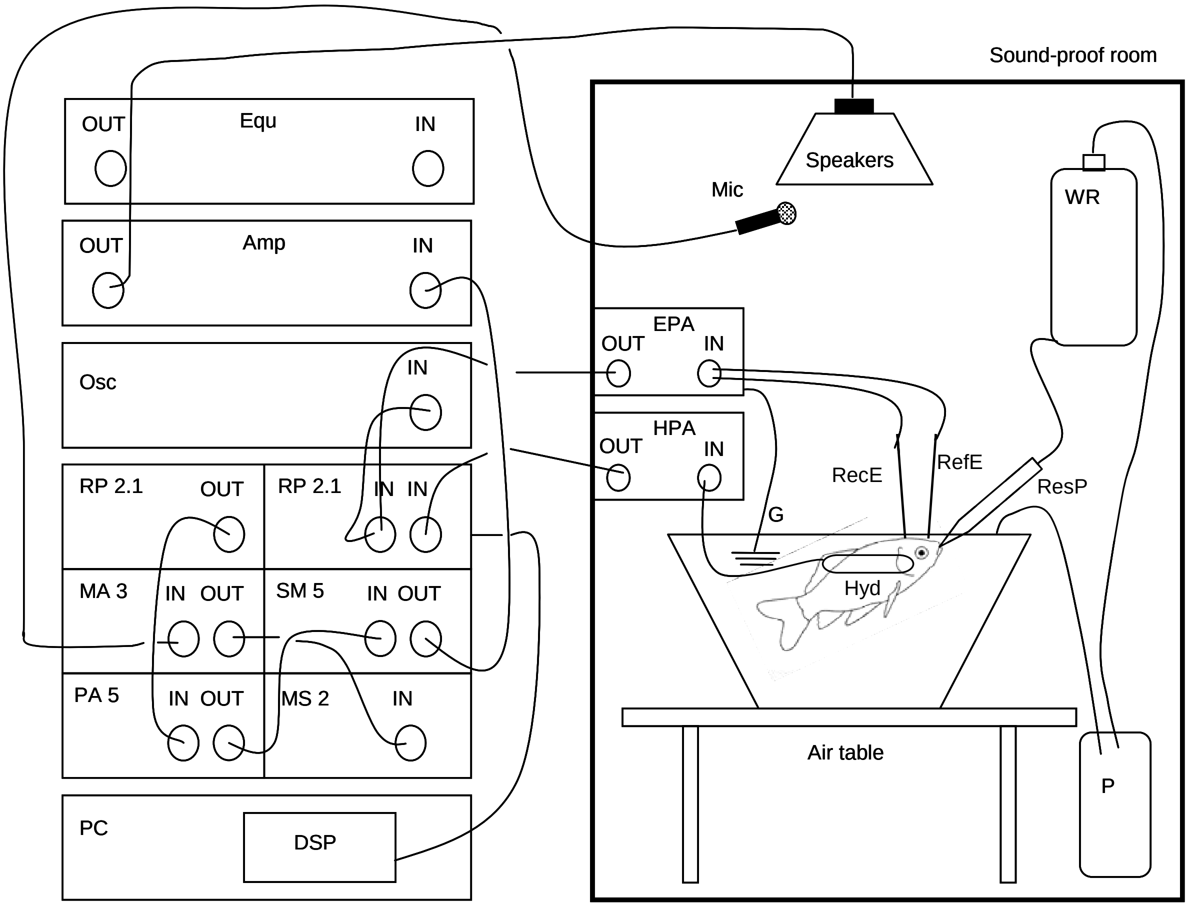

Fig. 3. Experimental setup for AEP-measurement. Abbreviations: Amp… Amplifier, DSP…Digital-Sound-Processor, EPA…Electrode-Preamplifier, Equ…Equalizer, G…Grounding, HPA… Hydrophone-Preamplifier, Hyd…Hydrophone, MA 3…Microphone-Amplifier, Mic…Microphone, MS 2…Microphone-Speaker, Osc…Oscilloscope, P…pump, PA 5…Programmable Attenuator, PC…Pentium 4 PC, RefE…Reference Electrode, RecE…Recording Electrode, ResP…Respiration Pipette, RP 2.1…Realtime-Processor, SM 5… Signalmixer, WR…Water reservoir

Both, sound stimulus presentation and AEP waveform recordings, were accomplished using a Tucker-Davis Technologies (TDT, Gainesville, FL, USA) modular rack-mount system (TDT System 3) controlled by a Pentium PC containing a TDT digital processing board and running TDT BioSig RP Software.

2.5. Sound stimuli

Sound stimuli waveforms and masking noise were created using TDT SigGen RP software and fed through a power amplifier (Alesis RA 300, Alesis Corporation, Los Angeles, CA, USA). A Subwoofer (Fostex PM0.5-Sub) and a Professional Studio Monitor (Fostex PM0.5 MK II), mounted 0.5 m above test subjects in the air, were used to present the stimuli during testing.

Sound stimuli consisted of tone bursts that were presented at a repetition rate of 21 per second. Hearing thresholds were determined at frequencies of 0.1, 0.3, 0.5, 0.8, 1, 2, 3 and 4 kHz. Frequencies were presented in a random order under normal laboratory conditions, and in the presence of continuous masking noise. The duration of sound stimuli increased from two cycles at 0.1 and 0.2 kHz, up to eight cycles at 4 kHz. Rise and fall times were one cycle at 0.1 and 0.2 kHz, and two cycles at all other frequencies. All bursts were gated using a Blackman window.

For each test condition, stimuli were presented at opposite polarities (180° phase shifted), and the corresponding AEPs averaged by the BioSig RP software in order to eliminate stimulus artefacts. The sound pressure level (SPL) of the tone bursts was reduced in 4 dB steps until the AEP waveform was no longer apparent. The lowest SPL, for which a repeatable AEP trace could be obtained, as determined by overlaying replicate traces, was considered the threshold (Kenyon et al., 1998).

A hydrophone (Brüel and Kjær 8101, Nærum, Denmark; frequency range: 1 Hz-80 kHz ± 2dB; voltage sensitivity: -184 dB re 1 V μPa^{-1}) was placed close to the right side of the animals (2 cm apart) in order to determine absolute SPL values underwater in the immediate vicinity to the subjects.

2.6. Masking noise

For playback of aquaria and pond noise during AEP recordings, 30 s of three aquaria noise recordings with the hydrophone in the middle of the aquaria were chosen: a) an external filter with vertical outflow 3 cm above the water surface (EFa), b) external filter with vertical outflow below the water surface (EFb) and c) with an internal filter (IF) with horizontal outflow below the water surface representing aquarium conditions (Fig. 1), and one recording of the pond noise (P) representing a typical seminatural habitat for fish kept for leisure.

Using external or internal filters with different outflow positions in comparison to the water surface typifies snapshots of the noise situation in aquaria, as the acoustic characteristics of filter systems tend to vary in dependence of the outflow position relative to the water surface. Nevertheless, the broad range of both, the level and spectral composition of the noise types chosen, fits our purpose to test the hearing abilities and the degree of masking in fishes kept for leisure in aquaria and ponds on some representative examples.

2.7. Statistical analysis

All audiograms obtained in the presence of the different noise types (lab-, pond- and three aquaria-noises) were compared by a two-factor analysis of variance (ANOVA) using a general linear model where one factor was masking noise and the other was frequency. The noise factor alone should indicate overall differences between masking conditions and in combination with the frequency factor if different tendencies exist at different frequencies of the audiograms. This was followed by Bonferroni's multiple comparison procedures to test among which noise conditions the audiograms differed from each other. In order to test for differences at the separate frequencies, hearing thresholds were compared using one-way ANOVAs followed by Bonferroni's post-hoc test. The level of significance was set at $P <$ 0.006 (Bonferroni correction by the number of tested frequencies).

Parametric statistical tests were applied because the data were normally distributed and showed homogeneity of variances. All statistical tests were run using SPSS 15.0.

3. Results

3.1. Diversity in noise levels and spectra

Continuous equivalent sound pressure levels ($L_{Leq,\ 1min}$) of aquaria and the ponds as well as noise spectra differed considerably (Tab. 1, Fig. 4).

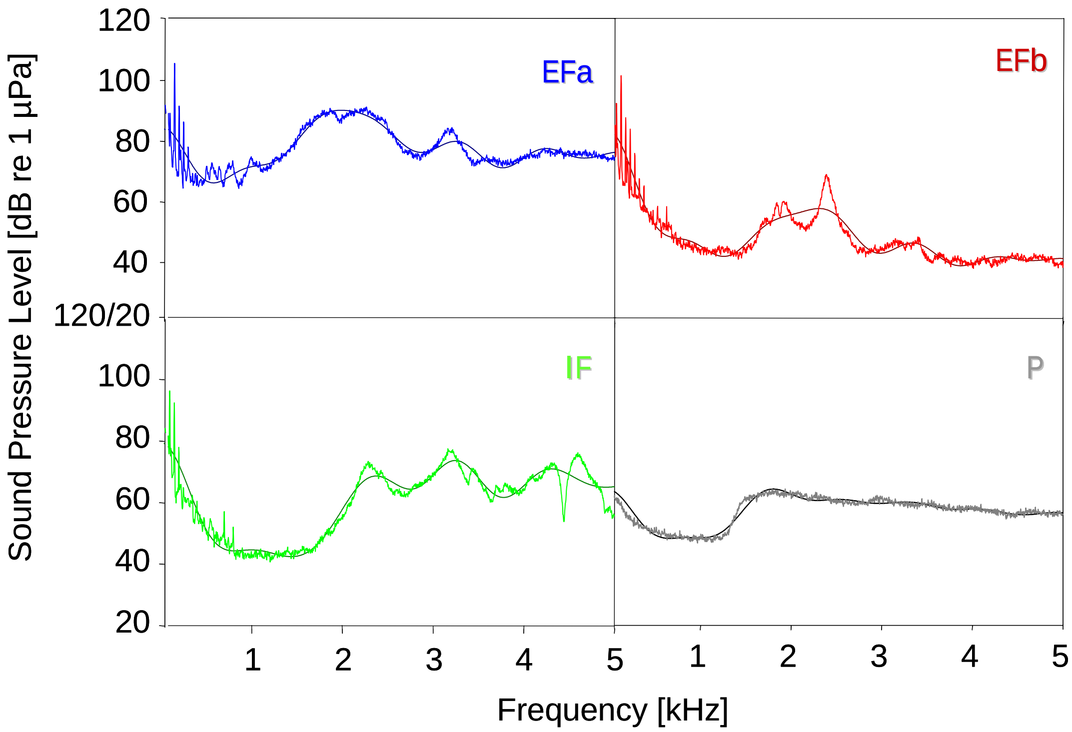

Fig. 4. Sound power spectra and cepstra of the different aquaria noise types and pond. EFa, external filter with outflow above water surface; EFb, external filter with outflow below surface; IF, internal filter; P, pond. Note the linear frequency axis scaling in this figure and the logarithmic scaling in Fig.s 5 and 7.

In the pond outside Vienna, the SPL of two different testing sites was almost identical (95.4 dB and 94.9 dB re 1 μPa $L_{Leq,\ 1\ min}$).

The investigations of diverse filter types and outflow setups in aquaria resulted in differences in noise levels. The SPL (L_{Leq}) of an internal filter with the outflow below the water surface increased with maximizing the aeration rate (114-119 dB). The external filter with the outflow of the plugged outlet pipe above the water surface was approximately 3 dB louder than with the outflow below the water surface. The SPL of the outflow of the outlet pipe with a plug at the end was higher than without such a plug (Tab. 1).

Table 1. Mean continuous equivalent sound pressure levels ($L_{Leq,\ 1min}$) of the pond at different places and of different aquaria noise types.

	SPL (L_{Leq})
pond (first position)	95.4
pond (second position)	94.9
IF (max. power, max. aeration)	117.2
IF (max. power, min. aeration)	117.2
IF (min. power, max. aeration)	119
IF (min. power, min. aeration)	113.5
EFa (outflow vertical to surface; with plug)	118.9
EFa (outflow 45° to surface; with plug)	116
EFa (outflow vertical to surface; without plug)	113.4
EFa (outflow 45° to surface; without plug)	113.9
EFb (outflow below surface; with plug)	114.2
EFb (outflow below surface; without plug)	113.9

Different locations of the external filter relatively to the aquaria were also investigated (Tab. 2). The SPL was lowest when the filter had no contact to the frame of the aquaria (111-113 dB). There was nearly no difference when the filter was located below the aquarium on 2 cm or 5 cm of styrofoam (113 dB) than standing right next to the aquaria on a wooden board or on a towel (114 dB).

Table 2. Mean continuous equivalent sound pressure levels ($L_{Leq,\ 1min}$) of different positions of the external filter relatively to the aquaria.

	SPL (L_{Leq})	
	EFa	EFb
below aquaria on 2 or 5 cm of styrofoam (same frame as aquaria)	113.3	112
on a chair (no contact with aquaria)	113.1	113.1
on the floor (no contact with aquaria)	111	110.7
on a wooden board (left of aquaria)	114	112
on a towel (left of aquaria)	113.5	112.4

Four noise types were chosen for investigating the masking effect on fishes. These types are frequently encountered by fish kept for leisure: noise types of aquaria with either an external filter with a vertical outflow above the water surface (EFa) and an external filter with outflow below the water surface (EFb), an internal filter (IF) with the outflow below the water surface with minimized power and aeration, and the noise of the pond (P) outside Vienna representing a semi-natural habitat.

The noise level in the pond (L_{Leq}) was about 20 dB lower than in the aquaria. Among the latter, EFa was the noisiest (119 dB), whereas the filters with the outflows below the water surface (IF, EFb) were quieter. Noise spectra show that all filters create a high amount of low frequency noise, but the spectral levels differed considerably at frequencies above 1.5 kHz (Fig. 4). EFa showed the highest spectral levels among all aquaria noise types (higher than 60 dB re 1 µPa) and a major noise boost from 1 to 2.5 kHz. EFb revealed a moderate decline towards higher frequencies, but featured also an energy raise like EFa from 1.5 to 3 kHz with a peak about 2.4 kHz (68 dB re 1 µPa). IF showed a similar decline as EFb until 1.5 dB, but then sound energy increased to 69 dB re 1 µPa. IF had at higher frequencies unsteady sound energy (54-70 dB re 1 µPa). The pond (P) outside Vienna representing a semi-natural habitat showed lower spectral levels than the aquaria-noise types in the low frequency range (<0.4

kHz), a broad noise window (about 50 dB re 1 µPa) and a quick increase of the SPL at 1.4 kHz. The pond noise revealed a flat, moderate decline towards higher frequencies.

3.2. Hearing under aquaria- and pond noise conditions

The baseline audiogram (measured under lab-noise conditions) for the goldfish showed greatest hearing sensitivity between 0.5 to 1 kHz, with hearing thresholds lower than 75 dB and a quick decline in sensitivity above 1 kHz. Comparing the baseline audiogram with the different masked audiograms by a two-factor ANOVA revealed overall significant differences between audiograms ($F_{4,200} = 2856$, $P < 0.001$) and a significant interaction between noise and frequency ($F_{28,200} = 69.4$, $P < 0.001$), yielding different effects of noise at different frequencies of the audiogram. The Bonferroni-adjusted *post-hoc* test showed that baseline audiograms were significantly different from each other filter noise type (EFa, EFb and IF) but not from the pond noise audiogram.

Playing back noise of the pond had no effect on the hearing thresholds at any of the frequencies tested, but noise from aquaria had pronounced effects on auditory sensitivity (Table 3, Fig. 5). In the low frequency range (0.1 and 0.3 kHz) thresholds did not differ in the presence of both EF noise types. At higher frequencies (0.8 and 1 kHz) thresholds with EFa were higher than with EFb noise. Hearing thresholds were masked by up to 20 dB (EFb at 0.1 kHz) and up to 24 dB (EFa at 0.5 kHz). In the presence of IF, the mean sensitivity at 0.1 and 0.3 kHz declined by maximally 10 dB whereas it decreased above 1 kHz up to 13 dB. In the best hearing range of goldfish (0.5, 0.8 and 1 kHz) the amount of threshold shift compared to the worse hearing range for EFb decreased and it increased for EFa and IF (Fig. 6).

Table 3. Hearing threshold values (dB re 1µPa) of *Carassius auratus* measured under the different background noise conditions. f, frequency; S.E.M., standard error of means; EFb, external filter with outflow below surface; EFa, external filter with outflow above water surface; IF, internal filter; P, pond.

	baseline	EFb	EFa	IF	P
f (kHz)	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.	Mean ± S.E.M.
0.1	87.33 ± 1.43	106.67 ± 2.08	102.50 ± 1.86	97.33 ± 1.48	87.67 ± 1.59
0.3	75.17 ± 2.30	93.50 ± 2.23	90.67 ± 2.23	82.67 ± 1.17	76.33 ± 2.33
0.5	68.00 ± 1.98	77.83 ± 2.47	85.50 ± 1.03	80.33 ± 1.50	68.33 ± 2.38
0.8	63.00 ± 2.25	71.50 ± 1.91	86.50 ± 2.08	81.50 ± 1.54	63.33 ± 2.47
1	69.17 ± 2.06	77.33 ± 1.48	87.83 ± 2.02	87.67 ± 1.12	70.17 ± 1.85
2	97.67 ± 2.14	112.50 ± 1.61	111.83 ± 3.11	110.17 ± 1.38	98.50 ± 1.93
3	105.50 ± 2.67	120.50 ± 2.38	121.33 ± 2.50	123.83 ± 1.66	106.83 ± 2.64
4	111.00 ± 2.58	119.50 ± 1.41	121.33 ± 2.17	122.33 ± 1.65	111.83 ± 1.52

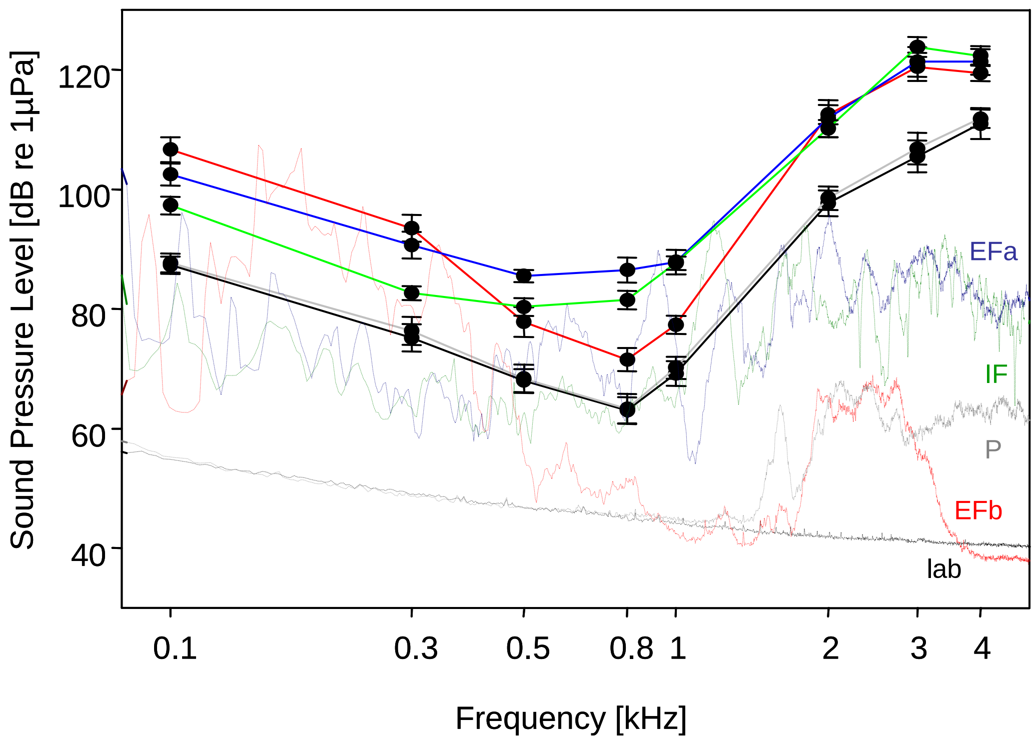

Fig. 5. Mean (± S.E.M.) hearing thresholds of *Carassius auratus* under laboratory conditions (baseline) and in the presence of the different artificial noise types (solid lines) and the sound power spectra of the corresponding noise types (broken lines). EFa, external filter with outflow above water surface; EFb, external filter with outflow below surface; IF, internal filter; P, pond.

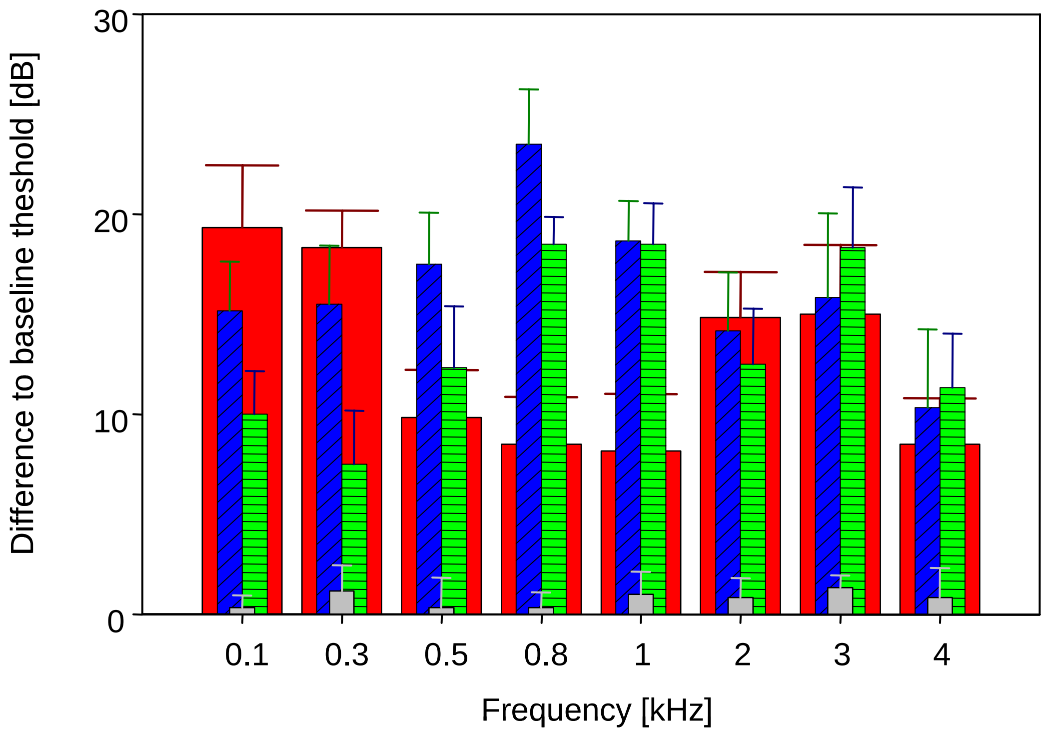

Fig. 6. Differences in hearing thresholds between the baseline audiogram and the masked audiograms. Values are means ± S.E.M. (*N*=6). Colours indicate the differences for the respective noise type according to Fig. 4. Red, EFb (external filter with outflow below surface); blue, EFa (external filter with outflow above water surface); green, IF (internal filter); grey, P (pond).

Threshold-to-noise (T/N) ratios were calculated by subtracting the spectrum level of noise (in a 1 Hz band) from the SPL at hearing threshold at this particular frequency for all thresholds. This calculation was made solely for masked thresholds that were significantly different from the baseline thresholds according to the one-way ANOVAs with Bonferroni post-hoc tests performed for each frequency. The mean T/N ratios ranged from 18.8 ± 1.3 dB to 47.3 ± 2.8 dB. The highest T/N ratios were measured at the highest test frequencies (Fig. 7).

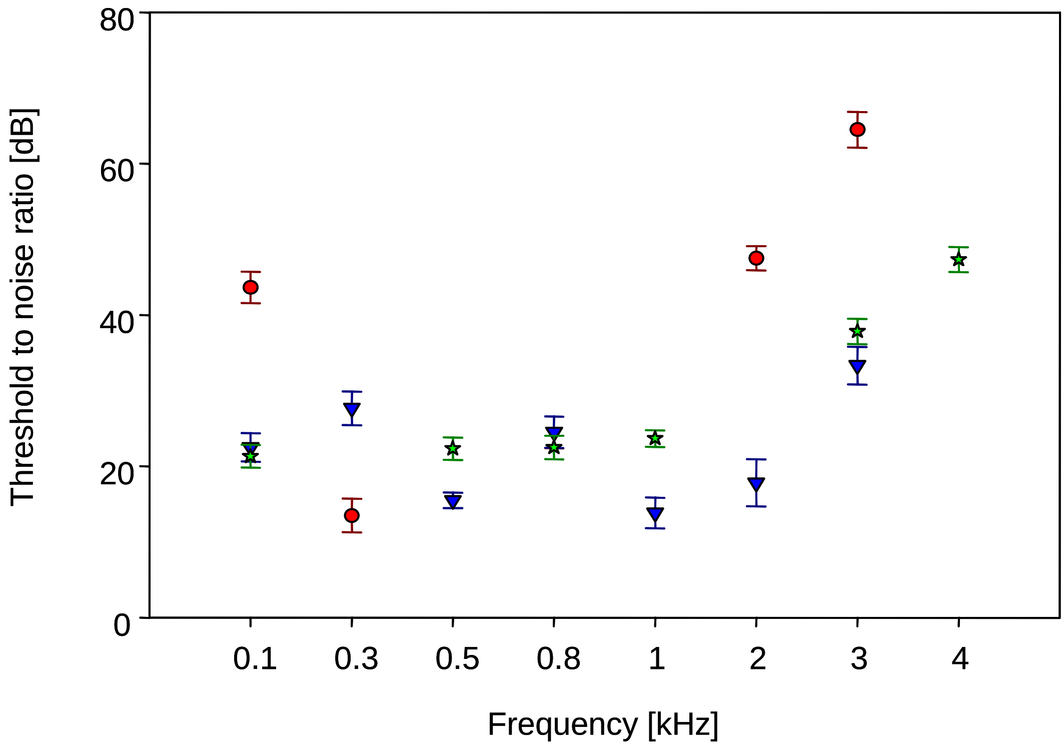

Fig.7. Mean (± S.E.M.) threshold-to-noise ratios for masked thresholds. (*N*=6). Colours indicate the T/N ratios for the respective noise types according to Fig. 4. Note that only masked thresholds that were statistically significantly different are represented.

4. Discussion

4.1. Diversity in noise conditions

Ambient noise in different habitats is highly diverse in terms of noise levels as well as energy distribution (Wysocki et al., 2007a). The natural habitats of hearing generalists have often relatively high ambient noise levels (Lugli and Fine, 2003). SPLs in creeks and streams are usually above 110dB re 1 µPa (L_{Leq}), whereas ambient noise levels in stagnant habitats with high percentages of hearing-specialized fish species such as backwaters and lakes are typically below 100 dB re 1 µPa (Wysocki et al., 2007a). This is consistent with current results, where in the pond outside Vienna the noise level was 95 dB re 1 µPa $L_{Leq,\ 1\ min}$.

Human-made holding conditions are often noisier than natural habitats. A wide range of waterborne noise was observed during the survey of underwater ambient noise measurements in aquaculture systems. Bart et al. (2001) found that mean broadband SPLs differed across various intensive aquaculture systems. These levels varied from <100 dB re 1 μPa in an earthen pond with the aerator turned off, 120 dB re 1 μPa RMS in concrete raceways, to 130 dB re 1 μPa in round fibreglass tanks of various sizes. They observed the highest noise levels in intensive recirculation culture systems with large (14 m-diameter x 4 m deep) fibreglass tanks (153 dB), and in a pond system with the aerator turned on (135 dB). Electric paddle wheel aerators contributed significantly to the noise levels in the outdoor ponds.

The noise level created in the aquarium by the internal filter with the outflow below the water surface increased with maximizing the aeration rate (114-119 dB). The external filter with the outflow of the outlet pipe with a plug at the rear end (so that the water did only exit the pipe via lateral holes) – above the water surface was louder than with the outflow below the water surface. The SPL of the outflow of the plugged outlet pipe was higher than without a plug, because numerous small water jets are louder than a large single outflow. This means, that the SPL will always be higher if more air gets into the water.

Furthermore higher frequency and more complex spectral components were observed in the aquaria with different filtering conditions than in the pond. SPLs of EFa above 0.5 kHz were higher than of EFb and IF because of more oscillating and collapsing air bubbles.

There was a maximum difference in noise levels of more than 40 dB between the spectral levels of EFa and the others (maximum difference between EFa and P ($\Delta_{EFa\text{-}P}$): 46 dB at a frequency of 0.15 kHz; $\Delta_{EFa\text{-}IF}$: 43 dB at 1.7 kHz; $\Delta_{EFa\text{-}EFb}$: 41 dB 1.6 kHz). The maximum difference of the noise levels in a concrete and fibreglass tank was about 15 dB at a frequency of 250 Hz (Terhune et al., 1990).

Low frequency noise is generated by water flows, ground vibrations, aquaria wall vibrations and electrical pumps and filter motors (Bart et al., 2001; Davidson et al. 2007). Lower frequency sound, below 0.1 kHz, with spectral levels 74-110 dB in the aquaria and 60-83 dB in the pond were detected. Bart et al. (Bart et al., 2001) measured SPLs below 400 Hz of 125-135 dB re 1 μPa.

In summary, minimizing the aeration of an internal filter with the outflow below the water surface decreased the SPL. In order to lower noise levels in aquaria, the following measures could be taken: (1) the water outflow pipe of the external filter should be close or below the water surface and not plugged. (2) If the outflow is above the water, it should not be vertically steered into the water (Tab. 1). (3) When using an external filter, it should have no contact to the table on which the aquaria is standing. It is proved advantageous to place the external filter below the aquaria on 2 cm or 5 cm of styrofoam (113 dB) than right next to the aquarium. Thus, using a quiet filter setup might help to reduce noise levels in the water.

4.2. Hearing under noise conditions

Hearing in our study animals was affected differently by the four noise conditions. The goldfish did not show masking effects during the presentation of pond noise. Noise levels in the pond were low (L_{Leq} < 100 dB) because there was no aerator or filtering system in this pond. A pond represents a seminatural habitat, in which goldfish and other cyprinids such as koi carps are often kept for leisure all over the world. The good hearing capabilities of otophysans are well adapted to quiet habitats, and these fish are able to detect low level sound produced by prey or food items and con- or heterospecifics (Amoser and Ladich 2005; Wysocki and Ladich 2005b).

Contrary to the pond, aquarium noise had pronounced effects on auditory sensitivity: Hearing in goldfish was heavily masked under all aquarium noise conditions. Hearing thresholds at every measured frequency were masked by at least 8 dB. In the low frequency range (0.1 and 0.3 kHz), hearing thresholds were highly masked because of the high amount of low frequency noise. In the best hearing range of goldfish (0.5, 0.8 and 1 kHz), the amount of threshold shift decreased when a more quiet water outflow e.g. below the surface was used; spectral analyses also showed a reduction of the spectral noise level in this frequency range.

The extent of threshold shift increased for EFa and IF noises because of the higher spectral noise levels caused by a greater quantity of aeration, oscillating and collapsing air bubbles. Above 1 kHz, all hearing thresholds were masked by about 13 dB because of the large amount of high frequency underwater noise generated by the water splashing noise as well as oscillating and collapsing air bubbles.

Hearing specialists are masked to a larger extent and they cannot exploit their excellent hearing abilities in environments with high SPLs (Popper and Fay, 1993; Scholik and Yan, 2001; Ladich and Popper, 2004; Amoser and Ladich, 2005; Scholz and Ladich, 2006; Wysocki et al. 2005a). These results indicate that hearing specialists are considerably masked under artificial holding conditions either in private aquaria or in aquaculture facilities. Wysocki et al. (2006) showed that cyprinids are susceptible to noise-induced stress response and hearing loss. This is probably the result of their hearing sensitivity and vulnerability to noise-induced hearing loss.

Hearing generalists lack accessory hearing structures (air-filled cavities connected to the inner ear) to enhance auditory abilities (Hawkins and Myrberg, 1983; Ladich and Popper, 2004). They therefore essentially respond to the particle motion component of low frequency sounds (and only below 1 kHz) at relatively high sound intensities. Generalists exhibit their best hearing range at lower frequencies than specialists, with worse hearing thresholds throughout the audiogram. According to prior findings (Wysocki and Ladich 2005b, Amoser and Ladich 2005) they would be only moderately or not at all masked in the presence of different aquarium noise types. Wysocki and Ladich (2005a) showed that masking was low in the presence of white noise. The European perch *Perca fluviatilis*, a non-specialist, was barely affected by quite different ambient noise types in any aquatic habitat due to its low hearing sensitivity (Amoser and Ladich, 2005). Similarly, no difference in auditory thresholds was found between rainbow trout *Oncorhynchus mykiss* reared in 115 dB tanks and 150 dB tanks (Wysocki et al., 2007b).

Anthropogenic noise is not only masking hearing under artificial holding conditions in aquaria and aquaculture facilities, it is also increasing in natural habitats of fishes and affects different fish species. Noise emanating from ships masks hearing in several non-related fish groups in several coastal regions. Low frequency ship noise masks hearing and subsequently the detection of conspecific sounds in representatives of Sciaenidae (*Sciaena umbra*) and Pomacentricae (*Chromis chromis*) in the Adriatic Sea (Codarin et al., 2008) and Batrachoididae (*Halobatrachus didactylus*) in the coastal regions near Lisbon (Vasconcelos et al. 2007). Thus, these two studies are the first indication that anthropogenic noise also impacts acoustic communication in representatives from marine fish families inhabiting the European coast. These and our results show that anthropogenic noise impairs hearing in fish not only in ponds or other freshwater habitats, but also in the sea.

Goldfish did not reproduce in our holding aquaria, but they spawn in the pond. The reason for differences in reproductive behaviour might be the lower noise level in the pond, lower population density and seasonal changes in temperature and light conditions. This is consistent with prior results where fat stores, growth, and several reproductive indices of the teleost fish, *Tilapia aurea* were influenced by a broad-band sound, at a level of 140 dB for only 20 min/d (Meier and Horsemann, 1977). The authors claimed that the daily photoperiod and the daily interval of noise either stimulated or inhibited growth depending on the specific schedule chosen. Also Banner and Hyatt (1973) showed that a 20 dB increase in sound in the 40 to 1000 Hz frequency range was significantly lethal during embryonic development of *Cyprinodon variegatus* and reduced viability of eggs and resulting larvae.

The mean T/N ratios of the hearing specialist *C. auratus* increased with increasing frequency. This trend has also been shown in hearing specialists such as the common carp *Cyprinus carpio*, the catfish *Platydoras costatus* and the topmouth minnow *Pseudorasbora* *parva*, as well as for generalists such as the European perch *Perca fluviatilis* and the Lusitanian toadfish *Halobatrachus didactylus* (Amoser and Ladich, 2005; Wysocki and Ladich, 2005b; Scholz and Ladich, 2006; Vasconcelos et al., 2007).

The T/N ratio is very important for understanding the influence of ambient noise on the detection of relevant signals. The ability to segregate important cues from a mixture of sound sources in the environment could be a major selective force in the evolution of hearing specializations. Fay (1998) showed in the goldfish the skill of detecting the temporal and the spectral characteristics of sound. This can be the reason, why stressful conditions have a detrimental impact on health and growth of specialists.

5. Conclusion

Fishes kept for leisure are exposed to different levels and spectra of background noise. We observed higher and more complex noise spectra in the filtered aquaria than in the pond. Low-frequency underwater noise was generated mostly by the motors of the filters, whereas high-frequency noise was probably due to oscillating and collapsing air bubbles (Bart et al. 2001).

Current data show that goldfish were heavily masked under artificial holding conditions and cannot exploit their excellent hearing abilities in environments with high noise levels. Aerators and other sources of sound in aquaculture systems might be setup in a way that has only a minimal effect on fish physiology (masking, stress) and growth. For decreasing the SPLs, the splashing of water should be minimized, the outflow of the outlet pipe should be close or below the water surface and no plug should be put into the end of the pipe. The filter should not be in contact with the table of the aquaria. If necessary it is advisable to place the filter below the aquaria on styrofoam or other soft material than standing right next to the aquaria.

Thus, using a quiet filter setup with a relatively quiet outflow might not compromise aeration of the water but help to improve holding conditions.

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Appendix

Hearing thresholds of *Carassius auratus* under laboratory conditions (baseline) and in the presence of the different artificial noise types and the sound power spectra of the corresponding noise types. EFa, external filter with outflow above water surface; EFb, external filter with outflow below surface; IF, internal filter; P, pond.

f (kHz)	0.1	0.3	0.5	0.8	1	2	3	4
baseline	92	78	67	64	73	97	103	114
	86	79	64	59	64	88	109	121
	90	81	72	72	75	100	100	110
	82	66	62	56	66	97	97	103
	86	76	75	62	73	102	110	106
	88	71	68	65	64	102	114	112
EFb	103	92	77	71	73	113	121	120
	107	94	68	68	74	111	120	125
	110	97	75	70	77	106	111	117
	115	87	79	67	78	113	126	119
	102	102	84	73	79	114	127	121
	103	89	84	80	83	118	118	115
EFa	103	92	85	80	92	107	122	117
	97	86	88	84	78	107	110	124
	107	89	81	89	89	102	126	122
	107	87	87	85	90	117	123	127
	104	101	87	95	89	122	127	125
	97	89	85	86	89	116	120	113
IF	101	83	77	84	87	107	122	122
	97	86	81	79	83	111	117	123
	97	78	78	84	89	107	124	127
	102	83	83	76	87	116	125	116
	94	85	77	80	89	109	129	126
	93	81	86	86	91	111	126	120
P	94	77	73	66	75	97	103	114
	84	77	66	59	70	90	109	116
	90	81	74	74	75	98	103	112
	84	66	60	57	66	102	99	105
	86	82	73	62	71	102	110	112
	88	75	64	62	64	102	117	112

Zusammenfassung

Vereinzelte Untersuchungen an Fischen zeigen, dass ihr Verhalten und die Hörempfindlichkeit durch Unterwasserlärm beeinflusst werden. Diese Studie konzentrierte sich auf die typischen Haltungsbedingungen von Fischen in Aquarien und Teichen. Die Lärmspektren zeigten, dass alle gemessenen Aquarienfilter einen großen Anteil an niederfrequenten Lärm und zusätzlich hochfrequenten Lärm durch den Wasserausstrom oberhalb der Wasseroberfläche produzierten. Audiogramme eines Hörspezialisten, des Goldfisches *Carassius auratus*, wurden zwischen 0.1 and 4 kHz mit Hilfe der nicht invasiven Ableitung akustisch evozierter Potentiale (AEP) ermittelt. Das Ausmaß der Maskierung wurde während vier verschiedene Lärmtypen untersucht: Aquarium mit Innenfilter mit Ausströmöffnung unterhalb der Wasseroberfläche ($L_{Leq,\ 1\ min}$ = 114 dB re 1 µPa), Außenfilter mit Ausströmöffnung oberhalb der Wasseroberfläche (119 dB), Außenfilter mit Ausströmöffnung unterhalb der Wasseroberfläche (115 dB) und ein Teich ohne Filterung (95 dB). Das Hören der Goldfische war in der Gegenwart von Lärm aller Filtertypen maskiert, vor allem bei 0.1 und 0.3 kHz während des Lärms des Außenfilters (Hörverschlechterung von 15-19 dB). Teichlärm hatte keinen Einfluss auf die Hörschwelle im Vergleich zu den leisen Laborbedingungen. Diese Ergebnisse weisen darauf hin, dass Hörspezialisten bei den untersuchten Haltungsbedingungen in Aquarien, vermutlich aber nicht in Teichen, beträchtlich maskiert waren. Aus diesem Grund sollte zur Verbesserung der Haltungsbedingungen in Aquarien eine leise Filteranlage mit einer leisen Wasserausströmung verwendet werden, die die Sauerstoffsättigung des Wassers nicht beeinträchtigt.